Phylogenetic studies of *Ephelis* species from various locations and hosts in Asia

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Ephelis japonica and *E. oryzae* are biotrophic fungi that form systemic epiphytic associations with warm-season grasses. *Ephelis* has been recognized as the anamorph of *Balansia* and *Myriogenospora*, and a synanamorph of *Atkinsonella*; all three genera belong to *Clavicipitaceae* (*Ascomycota*). The teleomorphs have not been detected for *E. japonica* and *E. oryzae*. *Balansia oryzae* had been regarded as the teleomorph of *Ephelis oryzae*, however, this teleomorphic name is not validly published for the teleomorph alone (Art. 59.6). We analyzed the sequences of the ITS1, ITS2 and 5.8S rDNA regions of 33 *Ephelis* isolates from Japan, Korea, China, Nepal and India. Phylogenetic relationships of these isolates were analyzed, together with other clavicipitaceous fungi for which sequences were obtained from GenBank. All Asian *Ephelis* isolates formed a single cluster, which comprised two subgroups. One subgroup had strong affinity with *B. andropogonis*, an epibiont of grasses in tropical regions of Asia. The second subgroup had strong affinity with *B. discoidea*, an epibiont of grasses in the Americas. A close relationship was also shown to *B. asperata*, another epibiont of grasses of tropical regions of Asia. Our results do not justify the separation of Asian *Ephelis* epibionts into *E. oryzae* and *E. japonica*. *E. japonica*, established in 1904, ten years prior to the name *E. oryzae*, is the appropriate name for the asexual *Ephelis* epibionts of warm season grasses of Asia.

INTRODUCTION

Warm-season grasses (*Poaceae*) in Japan and other parts of Asia are host to systemic fungi that have conidia characteristic of *Ephelis*. No sexual stage has been observed. Hyphae are present on the surface of stem apices, and with some host species, on the surface of leaf blades (Christensen *et al*. 2000). Dense hyphal growth, initially white but becoming black, covers inflorescences, preventing flowering. The presence of the stroma and the absence of seed production has given rise to the name black choke for the disease caused by *Ephelis* species in Japan. Hennings (1904) described the causal fungus of black choke disease as *E. japonica*.

An *Ephelis* infecting rice (*Oryzae sativa*) in India was named *E. oryzae*. (Sydow 1914). This fungus seriously reduced the yield of rice in India and also in China, with reductions of 30% reported in Yunnan province during 1940–1944 (Wei 1975). Improvement of rice cultivars and cultivation practices in recent years has almost completely eliminated this disease in both India and China, and plants infected with *E. oryzae* are rare. However, the potential of *E. oryzae* to reduce production cannot be dismissed.

Ephelis oryzae has been recorded on a wide range of grasses other than rice in both China (Tai & Siang 1948, Wei 1975), and India (Govinda Rao, Reddy & Venketa Reddy 1959, Govindu & Thirumalachar 1960). The reported host grasses of *E. oryzae* in China include species of *Eragrostis*, *Paspalum*, and *Setaria*. However, in Japan these grasses were reported as being host to *E. japonica* (Henmi 1928).

Ephelis is the anamorph of the teleomorphic genera *Balansia* and *Myriogenospora*, and a synanamorph of *Atkinsonella* (Diehl 1950), and is characterised by the presence of filamentous, hyaline, unicellular conidia. The three teleomorphic genera belong to the *Clavicipitaceae* (*Ascomycota*). Although no sexual stage has been confirmed on *Ephelis*-infected warm season grasses in Japan and East Asia, there are two reports of *Ephelis* as *Balansia* species. The first by Narasimhan & Thirumalachar (1943) as *B. oryzae*; however, this name is not available for the teleomorph as no Latin description or diagnosis and no description of asci or ascospores were supplied and has to be treated as a new combination typified by the anamorph (Art. 59.6) despite the placement in *Balansia*. The second was

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Ephelis sp. causing sterility of spiklets of *Aristida* that was referred to as the conidial stage of *B. andropogonis* (Patel, Gokhale & Kulkarni 1951).

In this paper we report on studies which investigated the relationship between Asian *Ephelis* isolates named as either *E. japonica* or *E. oryzae*, by comparing ITS1, ITS2 and 5.8S rDNA sequences of *Ephelis* isolates from a wide range of grasses and locations, and from clavicipitaceous species for which sequences were available from GenBank.

MATERIALS AND METHODS

Fungal isolates

Thirty two *Ephelis* isolates were obtained from 21 species of 14 genera of *Poaceae*, collected in Japan (5 sites), China (3 sites), Korea, and Nepal. Their hosts and locations are shown in Table 1; the latter include the subtropical island Ishigakijima (Okinawa, Japan), and the high altitude (2200 m) region of Ilam (Eastern Nepal). All voucher specimens and isolates are preserved at the herbarium of the Kyoto University Museum. We also examined *E. oryzae* isolate ATCC 15432 from rice in India. Included in the study were sequences of 36 isolates comprising 25 species of clavicipitaceous fungi from GenBank (Table 2). With some of these isolates, including those of *B. discoidea* and *B. asperata*, sequence information was available only from the ITS1 region.

DNA extraction

Fungal genomic DNA was extracted from liquid cultures in complete medium (0.15% $Ca(NO_3)_2 \cdot 4H_2O$, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.04% KH_2PO_4, 0.003% K_2HPO_4, 0.1% yeast extract, 0.1% tryptone, 1% glucose (w/v)) by the method of Nakada *et al.* (1994). All isolates were grown on a rotary shaker at 25 °C for 3 d, and the mycelia were collected by centrifugation (2 min, 15000 ***g***). The mycelia were macerated in 300 µl extraction buffer (50 mM Tris-HCl (pH 8.0), 125 mM EDTA, 100 mM NaCl, 2% (w/v) sodium *N*-lauroylsarcosinate, 1% (v/v) 2-mercaptoethanol) in 1.5 ml micro-centrifuge tubes. After extractions with phenol: chloroform: isoamyl alcohol (25:24:1), the nucleic acids were ethanol precipitated and treated with RNase. The DNA samples were stored in TE buffer at −20°.

PCR amplification and DNA sequencing

The ITS4 and ITS5 primers amplified the ITS1, 2 and 5.8S regions of rDNA, as described by White *et al.* (1990). For PCR, we used Taq polymerase (TAKARA, Japan) and a TAKARA PCR Thermal Cycler (TP-3000). The PCR products were separated using electrophoresis in 0.7% agarose gel (Sea Plaque GTG Agarose, FMC BioProducts, USA) and cut out. Agarase I (NewEngland BioLab) dissolved the excised agarose

Table 1. *Ephelis* species used in this study with their subgroups.

Isolate	Host Plant	Location	Subgroup	Accession no.*
paragrass	*Brachiaria mutica*	Japan, Ishigakijima	1	AB038567
2-1.himehi	*Chloris divaricata*	Japan, Ishigakijima	1	AB038571
H-himehi	*Chloris divaricata*	Japan, Ishigakijima	2	AB038583
S-higeshiba	*Chl. barbata*	Japan, Ishigakijima	2	AB038568
O-michi	*Chrysopogon aciculatus*	Japan, Ishigakijima	1	AB038566
3-1.bermuda	*Cynodon dactylon*	Japan, Ishigakijima	2	AB038572
Bermuda	*Cynodon dactylon*	Japan, Ishigakijima	1	AB038575
giant.bermu	*Cyn. pletostachyrus*	Japan, Ishigakijima	2	AB038565
cynodon	*Cyn.* sp.	Japan, Ishigakijima	2	AB038581
9-2.amehis	*Digitaria violascens*	Japan, Ishigakijima	2	AB038573
A-mehishiba	*Digitaria violascens*	Japan, Ishigakijima	2	AB038574
chigaya	*Imperata cylindrica*	Japan, Ishigakijima	2	AB038580
11-1.michi	*Melica onoei*	Japan, Ishigakijima	1	AB038584
S-kobie	*Paspalum orbiculare*	Japan, Ishigakijima	2	AB038569
Eph.Jka	*Eragostis ferruginea*	Japan, Kyoto1	2	AB038592
Eph.6ka	*Eragostis ferruginea*	Japan, Kyoto2	2	AB038594
Eph.6ch	*Pennisetum alopecuroides*	Japan, Kyoto2	2	AB038591
Eph.Jpa	*Pas. dilatatum*	Japan, Tochigi	2	AB038593
Eph.suzume	*Pas. thunbergii*	Japan, Tochigi	2	AB038570
Eph.Ska	*Era. ferruginea*	Japan, Kumamoto	2	AB038589
Eph.Sch	*Pen. alopecuroides*	Japan, Kumamoto	2	AB038588
C.eulalia	*Eulalia quadrinervis*	China, Beijing	2	AB038576
C.eulaliop	*Eulaliopsis* sp.	China, Beijing	1	AB038582
C.setaria	*Setaria viridis*	China, Beijing	2	AB038579
C.penni1	*Pen. alopecuroides*	China, Beijing	2	AB038577
C.penni2	*Pen. alopecuroides*	China, Beijing	2	AB038589
C.penni3	*Pen. alopecuroides*	China, Sichuang	2	AB038578
C.paspalum	*Pas.* sp.	China, Jiangxi	1	AB038586
C.eragro	*Era. ferruginea*	China, Jiangxi	1	AB038585
Kor.eragro	*Era. ferruginea*	Korea, Kyongju	2	AB038590
Nep.arundi	*Arundinacea* sp.	Nepal, Ilam	1	AB038596
Nep.eragro	*Era.* sp.	Nepal, Ilam	2	AB038595
Eph.oryzae	*Oryza sativa*	India (ATCC15432)	2	AB038564

* Sequences submitted to DDBJ/EMBL/GenBank.

Table 2. Clavicipitaceous fungi for which sequences were obtained from GenBank.

Isolate	Species	Accession no.
Atk.hypoxy	*Atkinsonella hypoxylon*	U57405
Bal.andro1	*Balansia andropogonis*	U89372
Bal.andro2	*B. andropogonis*	U89370
Bal.ascler	*B. asclerotiaca*	U89368*
Bal.aspera	*B. asperata*	U89375*
Bal.cyperi	*B. cyperi*	U89369
Bal.disc1	*B. dicoidea*	U89373*
Bal.disc2	*B. dicoidea*	U89374*
Bal.hennin	*B. henningsiana*	U57404
Bal.obtect	*B. obtecta*	U57402
Bal.pilula	*B. pilulaeformis*	AF065611
Bal.strang	*B. stranglans*	U57403
Cla.africa	*Claviceps africana*	AJ011590
Cla.purpur	*Cla. purpurea*	U57699
Cla.sorghi	*Cla. sorghicola*	AJ011591
Ech.tu.Aru	*Echinodothis tuberiformis*	U57667
Ech.tu.351	*Ech. tuberiformis*	U70325
Epi.amari1	*Epichloë amarillians*	U57664
Epi.amari2	*Epi. amarillians*	U57665
Epi.amari3	*Epi. amarillians*	L07129
Epi.amari4	*Epi. amarillans*	L07142
Epi.baconi	*Epi. baconii*	L07138
Epi.elymi	*Epi. elymi*	L07131
Epi.festuc	*Epi. festucae*	L07139
Epi.glyce1	*Epi. glyceriae*	L07136
Epi.glyce2	*Epi. glyceriae*	L07137
Epi.typh1	*Epi. typhina*	L07132
Epi.typh2	*Epi. typhina*	L07133
Epi.typh3	*Epi. typhina*	L20306
Myr.atram1	*Myriogenospora atramentosa*	U57406
Myr.atram2	*M. atramentosa*	U57407
Neo.lolii	*Neotyphodium loli*	L07130
Neo.e187	*N.* sp.	L07134
Neo.e41	*N.* sp.	L07140
Neo.uncin1	*N. uncinatum*	U57670
Neo.uncin2	*N. uncinatum*	L07135
P.cinerea1	*Parepichloë cinera*	AF003989*
P.cinerea2	*P. cinera*	AF003987*
P.cynodontis	*P. cynodontis*	AF003988*
P.sclerotica1	*P. sclerotica*	AF003986*
P.sclerotica2	*P. sclerotica*	AF003985*
P.sclerotica3	*P. sclerotica*	AF003984*

* ITS1 region sequences.

gel containing the PCR products. The purified ITS regions were cloned in plasmid vector pZErO™-2 (Invitrogen, The Netherlands) and sequenced by the Sanger Method using an ALFred DNA sequencer (Amersham Pharmacia Biotech). Sequencing was carried out using the Amersham sequencing kit (Thermo sequenase fluorescent labeled primer cycle sequencing kit with 7-deaza-dGTP) with fluorescent primers (Amersham Pharmacia Biotech) M13-20 (Cy5-CGACGTTG-TAAAACGACGGCCAGT) and M13-Rvs (Cy5-GAGCGG-ATAACAATTTCACACAGG).

Phylogenetic analyses

The sequence data were edited with the software package DNAsis–Mac (version 3.0, Hitachi Software Engineering, Tokyo). Phylogenetic analyses were made as previously described (Shimizu, Tanaka & Tsuda 1997). The sequences from Asian *Ephelis* isolates and clavicipitaceous fungi (sequences from GenBank; Table 2), in all 27 species represented by 72 isolates of *Atkinsonella*, *Balansia*, *Echinodothis*, *Ephelis*, *Epichloë*, *Myriogenospora*, *Neotyphodium*, *Parepichloë*, and an isolate of each of three *Claviceps* spp. as the outgroup, were aligned with CLUSTAL W (Thompson, Higgins & Gibson 1994). Phylogenetic trees were constructed by a neighbour-joining method (Saitou & Nei 1987). The distance matrix was calculated using DNADIST with Kimura 2-parameter method with NEIGHBOR (transition to transversion rate: 2.0). One thousand replicate bootstrap samplings were carried out with the software package PHYLIP (version 3.72; Felsenstein 1993), using SEQBOOT, NEIGHBOR, and CONSENSE.

RESULTS

All Asian *Ephelis* isolates clustered together in the ITS1-ITS2 analysis with the *Balansia* sequences available (Fig. 1). The Asian *Ephelis* isolates were grouped with only *B. andropogonis*. The Asian *Ephelis* isolates separated into two subgroups; one with sequences about 543 bp (subgroup 1) and the second of 538 bp (subgroup 2) in length (Fig. 3). These subgroups were represented in nearly all (977 of 1000) bootstrap replications. The maximum genetic distance between the two subgroups was only 0.0382 (isolates C-eulaliop of subgroup 1 and Nep.eragro of subgroup 2), and within each subgroup was 0.0071 (11-1-michi, O-michi, Nep.arundi and C-eulaliop in subgroup 1) and 0.0109 (Nep.eragro and Eph.suzume in subgroup 2).

Subgroup 1 included nine of the 33 *Ephelis* isolates and both *B. andropogonis* isolates. Five of the nine *Ephelis* isolates in this subgroup were from Ishigakijima, three were from China, and one from Nepal. Subgroup 2 included the *E. oryzae* isolate ATCC 15432, from rice, and the other 23 *Ephelis* isolates including the other nine isolates from Ishigakijima. Two species of grass on Ishigakijima, *Chloris divaricata* and *Cynodon dactylon*, were host to an isolate of both subgroups. *Eragrostis ferruginea* in Honshu, Japan, and in Korea was host to isolates of subgroup 2, while in Jiangxi, China, the isolate from this grass belonged to subgroup 1.

We further analyzed the Asian *Ephelis* isolates by comparison with *B. discoidea*, *B. asperata*, and other graminicolous *Clavicipitaceae* fungi, comparing only the ITS1 region as ITS2 sequences were not available for these species. Subgroup 1 and subgroup 2 were represented in nearly all (980 of 1000) bootstrap replications using the ITS1 sequences. *B. andropogonis* was again included in subgroup 1, and the two *B. discoidea* isolates within subgroup 2. An isolate of *B. asperata* was located within the Asian *Ephelis* cluster but fell outside the two subgroups.

Graminicolous *Clavicipitaceae* separated into two groups when the *Claviceps* species were used as the outgroup (Fig. 2): Asian *Ephelis* epibionts were included in one group along with species of *Atkinsonella*, *Balansia*, *Echinodothis*, *Myriogenospora* and *Parepichloë*; *Epichloë* and *Neotyphodium* species comprised the other group.

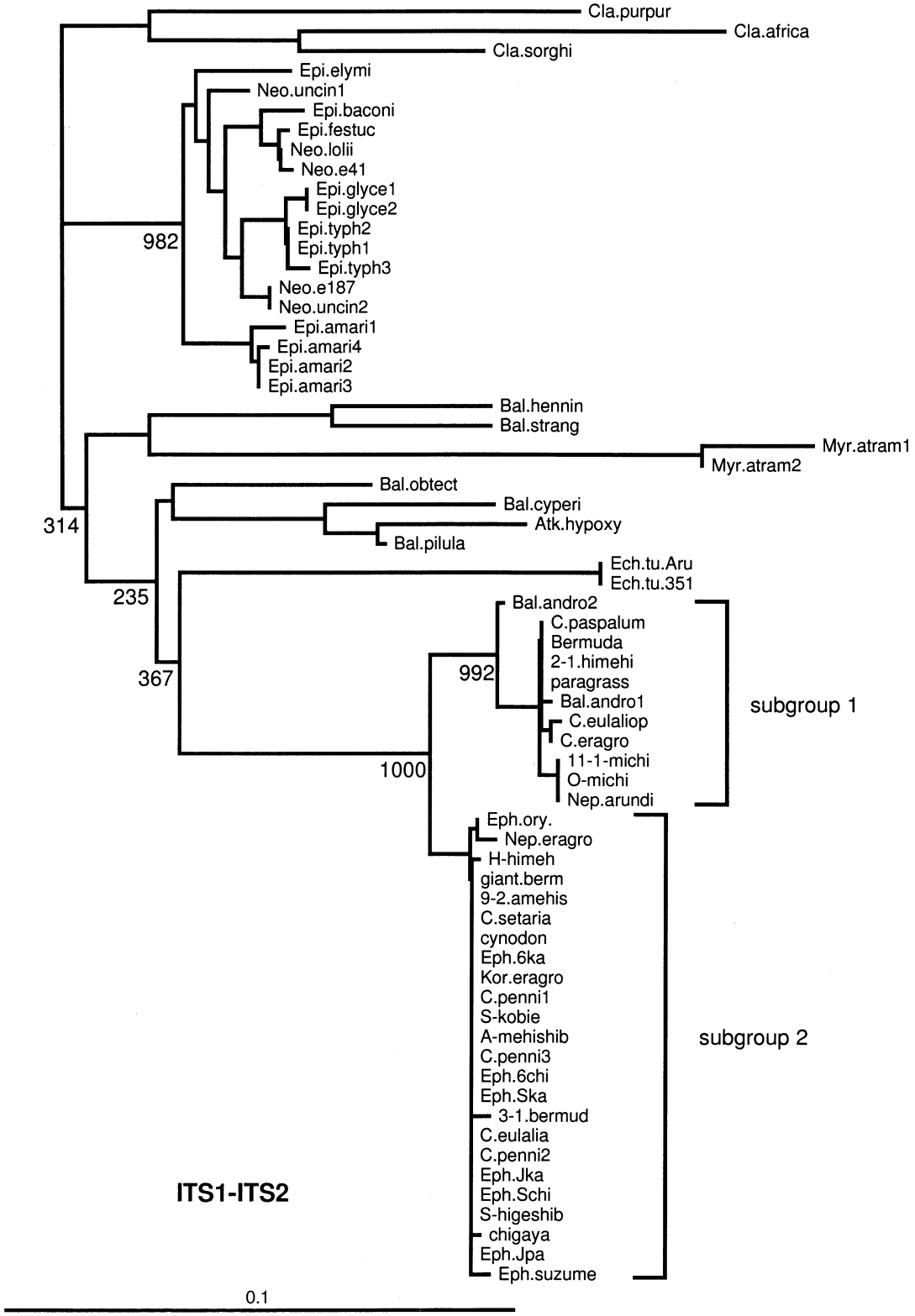

Fig. 1. Phylogram resulting from the neighbour-joining analysis of ITS1, 2 and 5.8S regions of rDNA of Asian *Ephelis* species and other clavicipitaceous fungi. The values shown at the nodes are the confidence levels from 1000 replicate bootstrap samplings. Bar = distance corresponding to 10 base changes per 100 nucleotide positions.

DISCUSSION

The phylogenetic analysis indicated that all of the Asian *Ephelis* isolates examined were closely related. The two subgroups present within the population examined had close affinities to *Balansia* species. Subgroup 1, with strong affinity to *B. andropogonis*, included isolates from the subtropical Ishigakijima, China, and Nepal. The second subgroup, with strong affinity to *B. discoidea*, also included isolates from Ishigakijima, China, and Nepal but in addition included isolates from the northern islands of Japan, Korea, and India. This second subgroup included the isolate from rice described

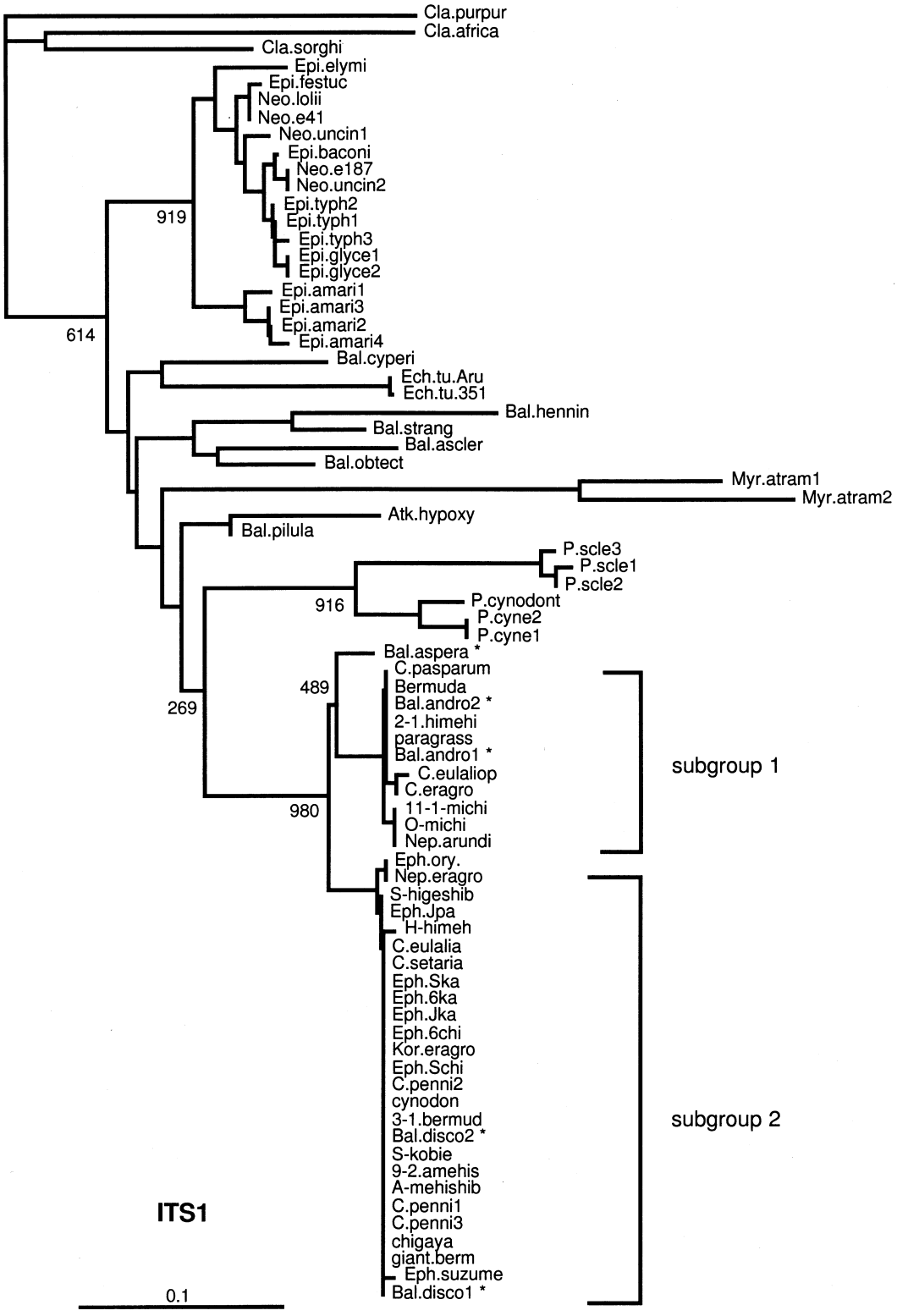

Fig. 2. Phylogram resulting from the neighbour-joining analysis of ITS1 region of Asian *Ephelis* species and other clavicipitaceous fungi. The values shown at nodes are the confidence levels from 1000 replicate bootstrap samplings. Bar = distance corresponding to 10 base changes per 100 nucleotide positions. * = teleomorphic species in the *Ephelis* group.

as *E. oryzae*. A third *Balansia*, *B. asperata*, also had strong affinities to the *Ephelis* isolates examined.

These three *Balansia* spp. form a single group, the *B. asperata* clade (Reddy *et al.* 1998); all are epibionts. *B. andropogonis* and *B. asperata* are Asian species, while *B. discoidea* is American. The two species with the strongest affinities to the Asian *Ephelis* epibionts, *B. andropogonis* and *B. discoidea*, have sessile ascomatal stromata, while those of *B. asperata* are stipitate. Differences have been reported in the subiculum of the three *Balansia* species of the *B. asperata* clade.

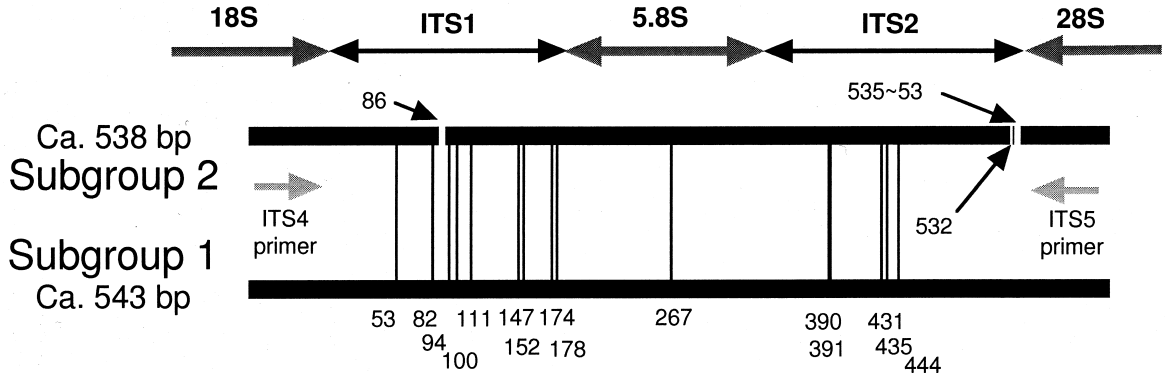

Fig. 3. The disparity of the two subgroups is due to insertions and/or deletions, and replacements. The ITS 1, 2 and 5.8S rDNA sequences of subgroups are 543 bp and 538 bp in length, respectively. An arrow indicates a deletion or insertion site. The numbers indicated by lines are replacement sites.

Those of *B. andropogonis* are white to grey and matted mycelial in texture, while those of *B. discoidea* are grey or black and smooth sclerotic, or smooth mycelial, in texture. The subiculum of *B. asperata* differs in being green to brown, with the texture flat matted mycelial. *B. andropogonis* is unique in having cupulate conidiomata (Reddy *et al.* 1998). A characteristic that distinguishes *B. andropogonis* from the other two species is the presence of cupulate conidiomata. In this study, cupulate conidiomata were observed on one Asian *Ephelis* specimen, *Arundinacea* sp. from Nepal, which belonged in subgroup 1, the subgroup which had a strong affinity to *B. andropogonis*.

Although the presence of two subgroups within the Asian *Ephelis* epibionts, each with close affinities to *Balansia* sp., may suggest the presence of two species, the evidence from this phylogenetic study cannot be considered as definitive. Supporting evidence could come from the examination of the stromata of isolates within each of the two subgroups, in particular, stromata of the different subgroups present on the same host species.

Diehl (1950) suggested the coidentity of both *Ephelis* species following examination of the hypothalli. Similarly, Phelps, Morgan-Jones & Owsley (1993) depicted little difference between the two species. The presence of the two subgroups from this phylogenetic study does not justify the separation of Asian *Ephelis* epibionts into *E. oryzae* and *E. japonica*. Although the isolate from rice, named as *E. oryzae*, was of subgroup 1, the current rarity of this fungus on rice resulted in just a single isolate being available to examine. It cannot be discounted that if additional isolates from rice were examined, both subgroups would be identified, as with the hosts *Chloris divaricata* and *Cynodon dactylon* on Ishigakijima. The name *E. japonica* was proposed in 1904 by Hennings, 10 years prior to the establishment of *E. oryzae*, and thus may be taken up for the asexual *Ephelis* that forms epiphytic associations with warm season grasses in Asia.

Each of the subgroups has a wide host range, with some grasses being confirmed as host to both. From the limited number of isolates examined in this study it is not possible to conclude that there is a difference in the parasitical specialization of each subgroup. Further, it is not known if isolates from one grass can form associations with those from other grasses. Our attempts to obtain infections were unsuccessful, but this lack of success is perhaps not surprising as efficient inoculation procedures have not been reported for *Balansia* spp. In one study, a wide range of inoculation procedures was completely unsuccessful in obtaining infection with *B. epichloë* (Rykard, Bacon, & Luttrell 1985).

Reddy *et al.* (1998) suggested the *Balansia* evolved in the Americas, the region with the bulk of the known species. Phylogenetic analysis based on the ITS1 region of rDNA revealed the presence of two clades within the *Balansia*, the *B. claviceps* and *B. asperata* clades. They concluded that the *B. claviceps* clade had evolved first, as the average number of base pair substitutions between nearest taxa was 15.6 bp substitutions, while for the *B. asperata* clade it was 8.0 bp. They suggested that the *B. asperata* clade might have arisen more recently through a colonization of tropical Asia. If this were true, they then suggested that the American species *B. discoidea* may either have been derived from a *B. andropogonis*-like ancestor that recolonized the Americas, or may have descended from the American ancestor of the *B. asperata* clade. From our finding that Asian *Ephelis* subgroup 2 has strong affinities with *B. discoidea*, it is possible that *B. andropogonis*, *B. asperata* and *B. discoidea* may have originated from an Asian *B. asperata*-like ancestor. The *B. discoidea* ancestor would then have become established later in the Americas, retaining its sexual stage, whereas the Asian *B. discoidea* lost its ability to produce the sexual stage and extended its range to include temperate zones.

The three *Balansia* spp. to which the Asian *Ephelis* epibionts are related, are found in tropical regions and thus the absence of the teleomorph in subtropical Asia may be a consequence of climate. Even in subtropical Ishigakijima the environmental conditions may not be suitable for the development of the teleomorph. However, the loss of the sexual stage of graminicolous *Clavicipitaceae* is not confined to Asian *Ephelis* epibionts. *Epichloë* species, which form endophytic associations with *Pooideae* grasses, also have asexual morphs, *Neotyphodium* spp. A feature of nearly all of these asexual morphs is that they are interspecific hybrids of *Epichloë* spp., or of *Epichloë* and *Neotyphodium* spp. (see Schardl & Philips 1997). The absence of sexual recombination has not prevented these fungi from being long-lived, successful symbionts. The

interspecific nature of *Neotyphodium* endophytes is not apparent from rDNA sequence analysis. No studies have been reported to determine if other asexual graminicolous *Clavicipitaceae* fungi are interspecific hybrids.

Asian *Ephelis* epibionts were grouped with species of *Atkinsonella*, *Balansia*, *Echinodothis*, *Myriogenospora*, and *Parepichloë* (Fig. 2). A similar result was also obtained by Kuldau *et al*. (1997). The *Parepichloë* species are epibiotic symbionts of warm-season grasses, and their ascomata are dark pigmented and flattened (White & Reddy 1998). Although no anamorph has been documented for *Parepichloë*, the above features are shared with some species in the *B. asperata* clade. Thus, *Parepichloë* species could be related to *Balansia* rather than *Epichloë* species, and one may justifiably speculate that at least some *Parepichloë* species may have an ephelitic state.

This study has revealed insights into the relationship between the asexual *Ephelis* epibionts which form associations with many warm season grasses in subtropical Asia, and the sexual *Balansia* spp. found in tropical Asia and Americas. However, many questions remain, and it will be through the combining of molecular techniques with morphological approaches that answers will be obtained.

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REFERENCES

Christensen, M. J., Takahashi, K., Kohno, K., Tsukiboshi, T. & Kobayashi, M. (2000) Occurrence of an *Ephelis* fungus on Ishigaki and observations on its epiphytic association with host grasses. *JIRCAS Journal* **8**: 49–59.

Diehl, W. W. (1950) *Balansia* and the *Balansiae* in America. [Agriculture Monograph No. 4]. United States Department of Agriculture, Washington DC.

Felsenstein, J. (1993) *PHYLIP* (*phylogeny inference package*). Version 3.572c. Department of Genetics, University of Washington, Seattle.

Govinda Rao, P., Reddy, G. S. & Venketa Reddy, T. C. (1959) Four new hosts of *Ephelis*. *Science and Culture* **25**: 74

Govindu, H. C. & Thirumalachar, M. J. (1960) Studies on some species of *Ephelis* and *Balansia* occurring in India I. Morphological account of some species of *Ephelis*. *Mycopathologia et Mycologia Applicata*, *Supplementum* **14**: 189–197.

Henmi, T. (1928) [Some plant disease fungi.] *Annals of the Phytopathological Society of Japan* **1**: 122. [In Japanese.]

Hennings, P. (1904) Einige Neue Pilze Aus Japan II. *Hedwigia* **43**: 150–153.

Kuldau, G. A., Liu, J.-S., White, J. F. Jr., Siegel, M. R. & Schardl, C. L. (1997) Molecular systematics of *Clavicipitaceae* supporting monophyly of genus *Epichloë* and form genus *Ephelis*. *Mycologia* **89**: 431–441.

Nakada, M., Tanaka, C., Tsunewaki, K. & Tsuda, M. (1994) RFLP analysis for species separation in the genera *Bipolaris* and *Curvularia*. *Mycoscience* **35**: 271–278.

Narasimhan, M. J. & Thirumalachar, M. J. (1943) Preliminary note on the perfect stage of *Ephelis oryzae* Syd. (*Balansia oryzae* (Syd.) comb. nov.). *Current Science* **12**: 276.

Patel, M. K., Gokhale, V. P. & Kulkarni, N. B. (1951) Addition to fungi of Bombay I. *Indian Phytopathology* **4**: 64–66.

Phelps, R., Morgan-Jones, G. & Owsley, M. R. (1993) Systematic and biological studies in the *Balansieae* and related anamorphs. V. The *Ephelis* anamorph of *Balansia aristidae*. *Mycotaxon* **49**: 91–105.

Reddy, P. V., Bergen, M. S., Patel, R. & White, J. F. Jr (1998) An examination of molecular phylogeny and morphology of the grass endophyte *Balansia claviceps* and similar species. *Mycologia* **90**: 108–117.

Rykard, D. M., Bacon, C. W. & Luttrell, E. S. (1985) Host relations of *Myriogenospora atramentosa* and *Balansia epichloë* (*Clavicipitaceae*). *Phytopathology* **75**: 950–956.

Saitou, N. & Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406–425.

Schardl, C. L. & Phillips, T. D. (1997) Protective grass endophytes. Where are they from and where are they going? *Plant Disease* **81**: 430–438.

Shimizu, K., Tanaka, C. & Tsuda, M. (1997) Cloning of *Brn*1, a reductase gene involved in melanin biosynthesis in *Cochliobolus heterostrophus*. *Journal of General and Applied Microbiology* **43**: 145–150.

Sydow, H. (1914) Beiträge zur Kenntnis der Pilzflora des südlichen Ostindiens-II. *Annales mycologici* **12**: 484–490.

Tai, F. L. & Siang, W. N. (1948) 'I-Chu-Xsiang' disease of rice caused by *Ephelis oryzae* Sydow in Yunnan. *Acta Agriculturae* **1**: 125–131.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680.

Wei, J.-C. (1975) [Manual of rice diseases.] Science Press, Beijing. [In Chinese.]

White, J. F. Jr. & Reddy, P. V. (1998) Examination of structure and molecular phylogenetic relationships of some graminicolous symbionts in genera *Epichloë* and *Parepichloë*. *Mycologia* **90**: 226–234.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal DNA for phylogenetics. In *PCR Protocols: a guide to methods and applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. Academic Press, New York.

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